APR 2 5 2003



1600

## ENTERED

RAW SEQUENCE LISTING

PATENT APPLICATION: US/10/037,677A

DATE: 04/22/2003 TIME: 14:28:34

Input Set : A:\GC560-D1-SEQLIST.txt

```
4 <110> APPLICANT: Schellenberger, Volker
 5
         Liu, Amy D.
         Selifonova, Olga V.
 8 <120> TITLE OF INVENTION: Directed Evolution of Microorganisms
11 <130> FILE REFERENCE: GC560-D1
13 <140> CURRENT APPLICATION NUMBER: US 10/037,677A
14 <141> CURRENT FILING DATE: 2001-10-23
16 <150> PRIOR APPLICATION NUMBER: US 09/314,847
17 <151> PRIOR FILING DATE: 1999-05-19
19 <160> NUMBER OF SEQ ID NOS: 17
21 <170> SOFTWARE: FastSEQ for Windows Version 4.0
23 <210> SEQ ID NO: 1
24 <211> LENGTH: 741
25 <212> TYPE: DNA
26 <213> ORGANISM: Escherichia coli
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30 atgaaccaga ttggtgcgca ctatgaaggc cacaagatca ttgagattgg tgccgttgaa
                                                                          120
31 gtggtgaacc gtcgcctgac gggcaataac ttccatgttt atctcaaacc cgatcggctg
                                                                          180
32 gtggatccgg aagcetttgg cgtacatggt attgccgatq aatttttgct cgataagccc
                                                                          240
33 acgtttgccg aagtagccga tgagttcatg gactatattc gcggcgcgga gttggtgatc
                                                                          300
34 cataacgcag cgttcgatat cggctttatg gactacgagt tttcgttgct taagcgcgat
                                                                          360
35 attocgaaga ccaatacttt ctgtaaggtc accgatagcc ttgcggtggc gaggaaaatg
                                                                          420
36 tttcccggta agcgcaacag cctcgatgcg ttatgtgctc gctacgaaat agataacagt
                                                                          480
37 aaacgaacgc tgcacggggc attactcgat gcccagatcc ttgcggaagt ttatctggcg
                                                                          540
38 atgaccggtg gtcaaacgtc gatggctttt gcgatggaag gagagacaca acagcaacaa
                                                                          600
39 ggtgaagcaa caattcagcg cattgtacgt caggcaagta agttacgcgt tgtttttgcg
                                                                          660
40 acagatgaag agattgcagc tcatgaagcc cgtctcgatc tggtgcagaa gaaaggcgga
                                                                          720
41 agttgcctct ggcgagcata a
43 <210> SEQ ID NO: 2
44 <211> LENGTH: 246
45 <212> TYPE: PRT
46 <213> ORGANISM: Escherichia coli
48 <400> SEQUENCE: 2
49 Met Thr Ala Met Ser Thr Ala Ile Thr Arg Gln Ile Val Leu Asp Thr
51 Glu Thr Thr Gly Met Asn Gln Ile Gly Ala His Tyr Glu Gly His Lys
               20
                                   25
53 Ile Ile Glu Ile Gly Ala Val Glu Val Val Asn Arg Arg Leu Thr Gly
55 Asn Asn Phe His Val Tyr Leu Lys Pro Asp Arg Leu Val Asp Pro Glu
57 Ala Phe Gly Val His Gly Ile Ala Asp Glu Phe Leu Leu Asp Lys Pro
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DATE: 04/22/2003

Input Set : A:\GC560-D1-SEQLIST.txt
Output Set: N:\CRF4\04222003\J037677A.raw

75 59 Thr Phe Ala Glu Val Ala Asp Glu Phe Met Asp Tyr Ile Arg Gly Ala 85 90 61 Glu Leu Val Ile His Asn Ala Ala Phe Asp Ile Gly Phe Met Asp Tyr 105 63 Glu Phe Ser Leu Leu Lys Arg Asp Ile Pro Lys Thr Asn Thr Phe Cys 115 120 125 65 Lys Val Thr Asp Ser Leu Ala Val Ala Arg Lys Met Phe Pro Gly Lys 135 67 Arg Asn Ser Leu Asp Ala Leu Cys Ala Arg Tyr Glu Ile Asp Asn Ser 150 155 69 Lys Arg Thr Leu His Gly Ala Leu Leu Asp Ala Gln Ile Leu Ala Glu 165 170 71 Val Tyr Leu. Ala Met Thr Gly Gly Gln Thr Ser Met Ala Phe Ala Met 72 180 185 73 Glu Gly Glu Thr Gln Gln Gln Gly Glu Ala Thr Ile Gln Arg Ile 74 195 200 75 Val Arg Gln Ala Ser Lys Leu Arg Val Val Phe Ala Thr Asp Glu Glu 215 220 77 Ile Ala Ala His Glu Ala Arg Leu Asp Leu Val Gln Lys Lys Gly Gly 78 225 235 79 Ser Cys Leu Trp Arg Ala 80 245 82 <210> SEQ ID NO: 3 83 <211> LENGTH: 1164 84 <212> TYPE: DNA 85 <213> ORGANISM: Escherichia blattae 87 <400> SEQUENCE: 3 88 atgagetate gtatgtttga ttatetggtt ecaaatgtga aettetttgg ecegggegee 60 89 gtttctgttg ttggccagcg ctgccagctg ctggggggta aaaaagccct gctggtgacc 120 90 gataagggcc tgcgcccat taaagacggt gctgtcgatc agaccgtgaa gcacctgaaa 180 91 gccgccggta ttgaggtggt cattttcgac ggggtcgagc cgaacccgaa agacaccaac 240 92 gtgctcgacg gcctggccat gttccgtaaa gagcagtgcg acatgataat caccgtcggc 300 93 ggcggcagcc cgcacgactg cggtaaaggc attggtattg cggccaccca cccgggtgat 360 94 ctgtacaget atgeeggtat egaaacacte accaaceege tgeegeecat tattgeggte 420 95 aacaccaccg ccgggaccgc cagcgaagtc acccgccact gcgtgctgac taacaccaaa 480 96 accaaagtaa aatttgtgat tgtcagctgg cgcaacctgc cttccgtctc cattaacgat 540 97 ccgctgctga tgatcggcaa gcccgccggg ctgaccgccg ccaccggtat ggatgccctg 600 98 acccaegegg tagaggeeta tateteeaaa gaegeeaace eggttaeega tgeetetget 660 99 attcaggcca tcaaactgat tgccaccaac ttgcgccagg ccgtcgccct ggggaccaac 720 100 ctcaaagccc gtgaaaacat ggcctgcgcc tctctgctgg ccgggatggc ctttaacaac 780 101 gccaacctgg gctatgttca cgccatggct caccagctgg gcggcctgta cgacatggcc 840 102 cacggggtgg cgaacgcggt cctgctgccc catgtctgcc gctataacct gattgccaac 900 103 ccggaaaaat ttgccgatat cgccaccttt atgggggaaa acaccaccgg tctttccacc 960 104 atggacgcag cggagctggc catcagcgcc attgcccgtc tgtctaaaga tgtcgggatc 1020 105 ccgcagcacc tgcgtgaact gggggtaaaa gaggccgact tcccgtacat ggcagaaatg 1080 106 gccctgaaag acggcaacgc cttctctaac ccgcgcaaag ggaacgaaaa agagattgcc 1140 107 gacattttcc gccaggcatt ctga 1164

109 <210> SEQ ID NO: 4

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Input Set : A:\GC560-D1-SEQLIST.txt

	0 <211> LENGTH: 387															
		<212> TYPE: PRT														
					Escherichia			bla	blattae							
				NCE: Ara		Phe	Asp	Tvr	Leu	Val	Pro	Asn	Val	Asn	Phe	Phe
116	1				5					10					15	
117 118	Gly	Pro	Gly	Ala 20	Val	Ser	Val	Val	Gly 25	Gln	Arg	Cys	Gln	Leu 30	Leu	Gly
119 120	Gly	Lys	Lys 35	Ala	Leu	Leu	Val	Thr 40	Asp	Lys	Gly	Leu	Arg 45	Ala	Ile	Lys
121 122	Asp	Gly 50	Ala	Val	Asp	Gln	Thr 55	Val	Lys	His	Leu	Lys 60	Ala	Ala	Gly	Ile
123			Val	Ile	Phe			Val	Glu	Pro	Asn		Lys	Asp	Thr	Asn
124						70					75∙					80
125 126	Val	Leu	Asp	Gly	Leu 85	Ala	Met	Phe	Arg	Lys 90	Glu	Gln	Cys	Asp	Met 95	Ile
127 128	Ile	Thr	Val	Gly 100	Gly	Gly	Ser	Pro	His 105	Asp	Cys	Gly	Lys	Gly 110	Ile	Gly
	Tle	Δla	Ala		His	Pro	Glv	Asn		Tur	Ser	Tur	Ala		Tla	Glu
130			115				_	120		_		_	125	_		
	Thr		Thr	Asn	Pro	Leu		Pro	Ile	Ile	Ala		Asn	Thr	Thr	Ala
132	C1	130	71.	Com	C1	17 o 1	135	7\ ~~~	111.0	C	77-1	140	mh	7	шь	T
	145	THE	Ата	ser	GIU	150	rnr	Arg	HIS	Cys	155		Thr	Asn	Thr	Lуs 160
	Thr	Lys	Val	Lys		Val	Ile	Val	Ser	-	Arg	Asn	Leu	Pro		Val
136	Ser	Tle	Asn	Asn	165 Pro	T.e.ii	T.e.u	Met	Tle	170	T.ve	Pro	Ala	G1 v	175	Thr
138	001	110	11511	180	110	Dea	шей	1100	185	Cry	Lyo	110		190	БСС	1111
139 140	Aľa	Ala	Thr 195	Gly	Met	Asp	Ala	Leu 200	Thr	His	Ala	Val	Glu 205	Ala	Tyr	Ile
	Ser	T.178		Δla	Aen	Pro	Val		Zen	Δl =	Sar	Λ1 >	Ile	Gln	<b>Δ</b> 1 =	Tlo
142	-	210					215		_			220				
	Lys 225	Leu	Ile	Ala	Thr	Asn 230	Leu	Arg	Gln	Ala	Val 235	Ala	Leu	Gly	Thr	Asn 240
		T.vs	Δla	Ara	Glu		Met	Δla	Cvs	Δla		T.011	Leu	Δla	C1v	
146	БСи	כעם	mu	1119	245	21011	1100	112.0	Cys	250	DCI	пец	neu	nia	255	Mec
147	Ala	Phe	Asn	Asn	Ala	Asn	Leu	Gly	Tyr	Val	His	Ala	Met	Ala		Gln
148				260				-	265					270		
149	Leu	Gly	Gly	Leu	Tyr	Asp	Met	Ala	His	Gly	Val	Ala	Asn	Ala	Val	Leu
150			275					280					285			
151	Leu	Pro	His	Val	Cys	Arg	Tyr	Asn	Leu	Ile	Ala	Asn	Pro	Glu	Lys	Phe
152		290					295					300	•			
		Asp	Ile	Ala	Thr		Met	Gly	Glu	Asn		Thr	Gly	Leu	Ser	Thr
154						310					315					320
155 156	Met	Asp	Ala	Ala	Glu 325	Leu	Ala	Ile	Ser	Ala 330	Ile	Ala	Arg	Leu	Ser 335	Lys
	Asn	Val	G] v	Tle		Gln	His	Len	Ara		Len	Glv	Val	Lvs		Ala
158				340					345					350		
159	Asp	Phe	Pro	Tyr	Met	Ala	Glu	Met	Ala	Leu	Lys	Asp	Gly	Asn	Ala	Phe

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Input Set : A:\GC560-D1-SEQLIST.txt

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171 <400> SEQUENCE: 5 172 atgagctatc gtatgtttga ttatctggtt ccaaatgtra acttctttgg cccgggegcc gttttetgttg ttggccagcg ctgccagctg ctggggggta aaaaagccct gctggtgacc gataagggcc ttgaggggta ttaaggggt cattttcgac ggggtcagac gaacccgaa agacccgaa agacccgaa agacccgaa agacccgaa agacccgaa agacccgaa agacccgaa agacccgaa agacccgaa agacccaac gggggtcgagc cgttgtgacc cattttcgac ggggtcagac cgaacccga agacccgaa agacaccaac gagcagtggg actggtgggt cattttcgac ggggtcagac cattgataat caccgtcggc cgtgaaaggc attggtattg cggaaccacc accagcggtgat atgacggca cgggaaccacc accaacccg tgcggcaaccac accaacccg tgcgggtgat accaacccg cgggaaccac cagcgaagtc accaacccg tgcggcaaccac accaaccaaa accaaccag gggagcgaaccaac tgcgcaacctg tgcaaccacc gggggtgat accaaccaca gggagccaacc caccaaccaaa accacacaaa accacacaaga caccaacca	60 120 180 240 300 360 420 480 540 600 660 720 780 840 900 960 1020 1080 1140 1164											
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198 <400> SEQUENCE: 6 199 Met Ser Tyr Arg Met Phe Asp Tyr Leu Val Pro Asp Val Asp Phe Phe												
200 I 10 15 201 Gly Pro Gly Ala Val Ser Val Val Gly Gln Arg Cys Gln Leu Leu Gly												
202 20 25 30 203 Gly Lys Lys Ala Leu Leu Val Thr Asp Lys Gly Leu Arg Ala Ile Lys												
40.												
205 Asp Gly Ala Val Asp Gln Thr Val Lys His Leu Lys Ala Ala Gly Ile 206 50 60												
207 Glu Val Val Ile Phe Asp Gly Val Glu Pro Asn Pro Lys Asp Thr Asn 208 65												
209 Val Leu Asp Gly Leu Ala Met Phe Arg Lvs Glu Gln Cvs Asp Met Ile												
210 85 90 95 211 Ile Thr Val Gly Gly Ser Pro Leu Asp Cys Gly Lys Gly Ile Gly 212 100 105 110												

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Input Set : A:\GC560-D1-SEQLIST.txt

Output Set: N:\CRF4\04222003\J037677A.raw

213 214	Ile	Ala	Ala 115	Thr	His	Pro	Gly	Asp 120	Leu	Tyr	Ser	Tyr			Ile	Glu
215 216	Thr	Leu	Thr		Pro	Leu	Pro	Pro		Ile	Ala			Thr	Thr	Ala
217	Gly	130 Thr		Ser	Glu	Val	135 Thr	Arg	His	Cys	Val	140 Leu	Thr	Asn	Thr	Lys
219	147					150 Val					155					160
220					102	Leu				170					175	
222				TRO					185					190		
224			195			Asp		200					205			
220		210				Pro	215					220				
227 228	Lys 225	Leu	Ile	Ala	Thr	Asn 230	Leu	Arg	Gln	Ala	Val 235	Ala	Leu	Gly	Thr	
229 230	Leu	Lys	Ala	Arg	Glu 245	Asn	Met	Ala	Cys	Ala	Ser	Leu	Leu	Ala		240 Met
231	Ala	Phe	Asn	Asn		Asn	Leu	Gly	Tyr	250 Val	His	Ala	Met	Ala	255 His	Gln
232				260		Asp			265					270		
234			2/5			Arg		280					285			
236		290					295					300				
238	305					Phe 310					315					320
240					325	Leu				330					335	
241 242	Asp	Val	Gly	Ile 340	Pro	Gln	His	Leu	Arg 345	Glu	Leu	Gly	Val	Lys 350	Glu	Ala
243 244	Asp	Phe	Pro 355		Met	Ala	Glu	Met	Ala	Leu	Lys	Asp		Asn	Ala	Phe
245	Ser	Asn		Arg	Lys	Gly	Asn	360 Glu	Lys	Glu	Ile	Ala	365 Asp	Ile	Phe	Arg
246 247		3/0					375					380				_
248 250		> SE	ο τρ	NO:	7											
250 <210> SEQ ID NO: 7 251 <211> LENGTH: 12																
252 253					Δrti	fici	-1 C	^~								
255	253 <213> ORGANISM: Artificial Sequence 255 <220> FEATURE:															
256 <223> OTHER INFORMATION: wild type mutD gene																
258 <400> SEQUENCE: 7																
259 atgaccgcta tg																
261 <210> SEQ ID NO: 8 262 <211> LENGTH: 11																
263	<212	> ጥጥ	PE•	DИД • . т Т												
264	<213:	> OR	GANT:	SM:	Arti	fici	al c	amie	nce							
266 <	<220:	> FE	ATIIR	• 1 E•		~= 0.10	AT 0	-que	1100							

266 <220> FEATURE:

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VERIFICATION SUMMARY

PATENT APPLICATION: US/10/037,677A

DATE: 04/22/2003 TIME: 14:28:35

Input Set : A:\GC560-D1-SEQLIST.txt